Clinical and laboratory observations following oral or intramuscular administration of a live attenuated hepatitis A vaccine candidate

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Clinical observations made after immunising volunteers with a live attenuated hepatitis A vaccine are described. The candidate vaccine was prepared with the HM175 strain of hepatitis A virus and shown to be safe, immunogenic and efficacious in experimental animals. When the candidate vaccine was tested by oral administration in humans at increasing doses – 10^4, 10^5, 10^6 and 10^7 median tissue culture infective doses ($TCID_{50}$) – an antibody response was not observed at any dose. Volunteers who received similar doses by the intramuscular route developed antibody to hepatitis A three weeks after immunization with 10^6 or 10^7 $TCID_{50}$. The antibody response was sustained for the 12 weeks of the observation period. All volunteers remained healthy with normal results from liver tests throughout the monitoring period. Further clinical observations of this product are in progress.

Keywords: *Hepatitis A; viral hepatitis; liver*

INTRODUCTION

Although inactivated hepatitis A vaccines are in the process of preparation, logistical problems such as multiple injection and cost of the product could be significant impediments to implementing a worldwide immunization programme. A live, attenuated hepatitis A vaccine, if safe and immunogenic, would be a candidate to use in massive eradication programmes. Investigators at the National Institutes of Health in Maryland and investigators at SmithKline Beecham Biologicals, Belgium prepared the present candidate vaccine. Investigators from the US Army performed the first human trial of this preparation. Details of this trial are described.

MATERIAL AND METHODS

Study group

The study was approved by Institutional Review Committees. Before enrollment, informed consent was obtained from each volunteer.

In this clinical trial, volunteers received increasing titres of a live, attenuated hepatitis A vaccine by the oral route. Later, a second group of volunteers received similar doses by the intramuscular route. Eight volunteers received the live attenuated hepatitis A vaccine by the oral route in the following manner: two received a 10^4 median tissue culture infective dose ($TCID_{50}$), two received 10^5 $TCID_{50}$, two received 10^6 $TCID_{50}$ and two received 10^7 $TCID_{50}$. Six volunteers received the vaccine by the intramuscular route in the following manner: two received 10^5 $TCID_{50}$, two received 10^6 $TCID_{50}$ and two received 10^7 $TCID_{50}$

Candidate vaccine

The vaccine was prepared with the HM175 strain of hepatitis A virus (HAV). HAV was first propagated in African green monkey kidney cells (AGMKC) and then adapted to human MRC-5 fibroblast cells. Attenuation was attained by serial blind passages. An earlier publication described the vaccine preparation in AGMKC and pre-clinical studies in chimpanzees and marmosets[1]. The MRC-5 adapted vaccine candidate used in this study was also tested in chimpanzees and marmosets. These preclinical studies demonstrated that the vaccine was safe, immunogenic and efficacious in experimental animal models.

The work was performed at the following institutions: †Division of Medicine, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21702, USA. ‡National Institutes of Health, Bethesda, MD, USA. §Walter Reed Army Institute of Research, Washington, DC, USA. ¶Food and Drug Administration, Rockville, MD, USA. ¤SmithKline Beecham Biologicals, Rixensart, Belgium. *To whom correspondence should be addressed

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Clinical monitoring

Volunteers were admitted to a closed clinical ward at the US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland. Each volunteer remained on the ward for three days after immunization. Local or systemic side effects were monitored during the admission period and for 12 weeks following the immunization. Volunteers were asked to return at 8 and 12 months for serological follow-up. Vaccine was administered as a single dose of 1.0 ml orally or intramuscularly in the deltoid area.

Collection and testing of clinical samples

Sera were obtained prior to immunization and once a week for the next 12 weeks. In volunteers who completed the appropriate follow-up time, sera were also obtained at 6 and 12 months after initial administration of vaccine. Serum specimens were tested for alanine aminotransferase (ALT) and antibody to hepatitis A. ALT was tested with a Kodak EKTA Chem 700XR analyser (Rochester, NY, USA); normal values were 0–50 IU/ml. Antibody to hepatitis A was tested by four different methods, including a commercial radioimmunoassay (HAVAB, Abbott Laboratories, North Chicago, IL, USA) and an enzyme-linked immunoassay developed by SmithKline Beecham Biologicals (SKB-ELISA), which was more sensitive than the standard HAVAB, in which a level of $\geq$ 20 mIU/ml was considered positive. Selected sera were tested by the radioimmunofocus inhibition test (RIFIT) for neutralizing antibody to hepatitis A. With this test, a serum titre of $\geq$ 1:10 was considered positive[2]. In the fourth method, sera were tested for immunoglobulin M (IgM) anti-HAV by commercial radioimmunoassay (HAVAB-M, Abbott Laboratories).

Stools were collected from the volunteers two to three times per week for the first 12 weeks and were tested for the presence of hepatitis A virus by radioimmunoassay[3] and molecular biology techniques[4].

RESULTS

All volunteers remained healthy during the follow-up period (12 weeks to one year). No systemic complaints were present immediately after immunization or during long-term follow-up. Serum ALT levels remained normal in all 14 individuals during the period of observation.

Antibody to hepatitis A was not observed in any of the eight volunteers who received the vaccine by the oral route or in the two volunteers who received 10^5 $TCID_{50}$ by the intramuscular (i.m.) route. The four volunteers who received higher doses of vaccine i.m. (10^6 $TCID_{50}$ or 10^7 $TCID_{50}$) all had detectable antibody by ELISA as early as 3 weeks after immunization. Detectable levels persisted for the 12 weeks of observation. *Figure 1* shows the immune response of one of the volunteers who received 10^6 $TCID_{50}$ and another who received 10^7 $TCID_{50}$ vaccine. Selected sera tested for neutralizing antibody had titres ranging from 1:10 to 1:40 in the volunteer who received a 10^6 dose and 1:40 to 1:2560 in the volunteer who received a 10^7 dose. The time of appearance of neutralizing antibody is also depicted in *Figure 1*. The commercial HAVAB assay detected anti-HAV in only one of the volunteers, who received the 10^7 dose. IgM anti-HAV was not detected in any of the volunteers who received the vaccine orally. Sera from volunteers who received 10^7 $TCID_{50}$ i.m. had detectable IgM anti-HAV.

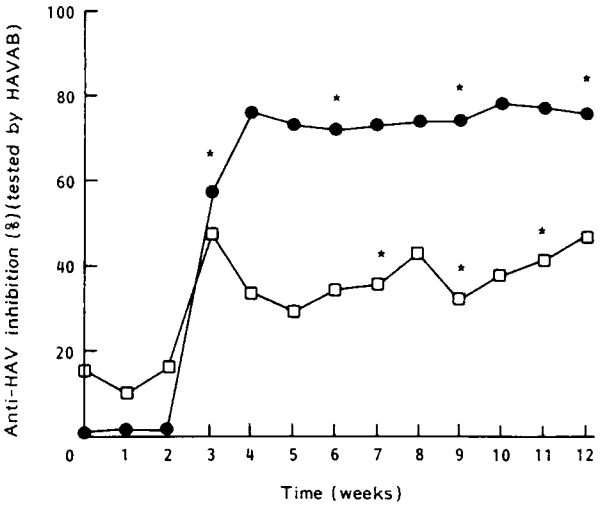

Figure 1 Antibody to hepatitis A following immunization with live, attenuated vaccine. Antibody to hepatitis A was tested by standard HAVAB (●, □) and neutralizing antibody (*). Vaccine was administered at time zero. □, 10^6 $TCID_{50}$; ●, 10^7 $TCID_{50}$. Neutralizing antibody was present at various titres (see text)

Stools from all volunteers who received the oral vaccine were negative for hepatitis A virus, while those from volunteers who have received the vaccine by the intramuscular route are in the process of being tested.

DISCUSSION

Although only a small number of volunteers received the vaccine orally, the vaccine was not immunogenic in them. This is probably due to overattenuation of the virus, although other causes, such as inactivation in the gastrointestinal tract or too small an inoculum, should be considered. The vaccine was safe and immunogenic by the intramuscular route at doses of 10^6 and 10^7 $TCID_{50}$. The antibody response was prompt: anti-HAV was observed within 3 weeks of immunization, persisted during the period of observation and did not diminish in titre. Such a response to a single inoculation of a preparation which lacked an adjuvant is remarkable. The presence of IgM anti-HAV in volunteers who received 10^7 $TCID_{50}$ without evidence of hepatitis is suggestive of asymptomatic replication of the virus. Final evaluation must await the results of attempts to detect HAV in stools of the volunteers.

Two other groups of investigators have reported clinical observations after immunizing volunteers with live attenuated hepatitis A vaccines. Provost *et al.*[5] described the F and F′ variants of the CR326 hepatitis A virus strain. While the F variant was highly immunogenic, it also caused abnormal serum ALT levels in a substantial proportion of individuals. The F′ variant was more attenuated: 10 of 11 volunteers had demonstrable anti-HAV by modified HAVAB without abnormal ALT levels when the vaccine candidate was administered by the subcutaneous route.

Another recent publication from this group of investigators has described further work with the F′ variant[6].

They observed that the immunogenicity of the product is dose-dependent, $10^{7.3}$ $TCID_{50}$ evoked an antibody response in 100% of the volunteers within 9 weeks after immunization. Lower doses were immunogenic in a smaller percentage of volunteers and anti-HAV was observed 4 to 6 months after immunization.

Chinese investigators have recently described studies of a live attenuated hepatitis A vaccine prepared from the H2 strain of HAV[7]. Twelve volunteers received the vaccine by the subcutaneous route. All volunteers remained healthy and had detectable anti-HAV by week 3 after inoculation. Follow-up of these individuals showed persistence of antibody one year after initial inoculation. Three subjects had detectable hepatitis A virus in the stool on days 8 to 21 after inoculation.

The present work, as well as the previous reports, strongly support the concept of a live, attenuated hepatitis A vaccine. Such a vaccine could have significant impact on the eradication of disease. It could be anticipated that a live, attenuated vaccine which requires minimal purification and no adjuvant would be less costly than currently available inactivated hepatitis A vaccines. If anti-HAV persists for a long time after one dose, the logistics of administration of this product would be simpler and more successful than with present hepatitis A vaccines.

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